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SYNTHESIS OF CHOLESTEROL AND ITS ANALOGS WITH FLUORINATED SIDE-CHAINS

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SUMMARY

Dithionite initiated addition of perfluoroalkyl iodide to an olefinic double bond proved to be an efficient method for the introduction of a perfluoroalkyl group to a steroid side chain. Starting from 3α, 6α - dihydroxy- 5β - cholanic acid, the synthesis of cholesterol and its analogs with a partially fluorinated side-chain (**6a**, $R_f = CF_2CF(CF_3)_2$; **6b**, $R_f = (CF_2)_2CF(CF_3)_2$; **6c**, $R_f = (CF_2)_3CF_3$; **6d**, $R_f = (CF_2)_4Cl$; **6e**, $R_f = (CF_2)_6Cl$; **6g**, $R_f = (CF_2)_6H$) and bis-sterol (**10**) was achieved in good yield. These compounds are not degraded by some microbial organism systems.

INTRODUCTION

Syntheses of fluorine-containing steroids for drug and other applications have been studied extensively over the past

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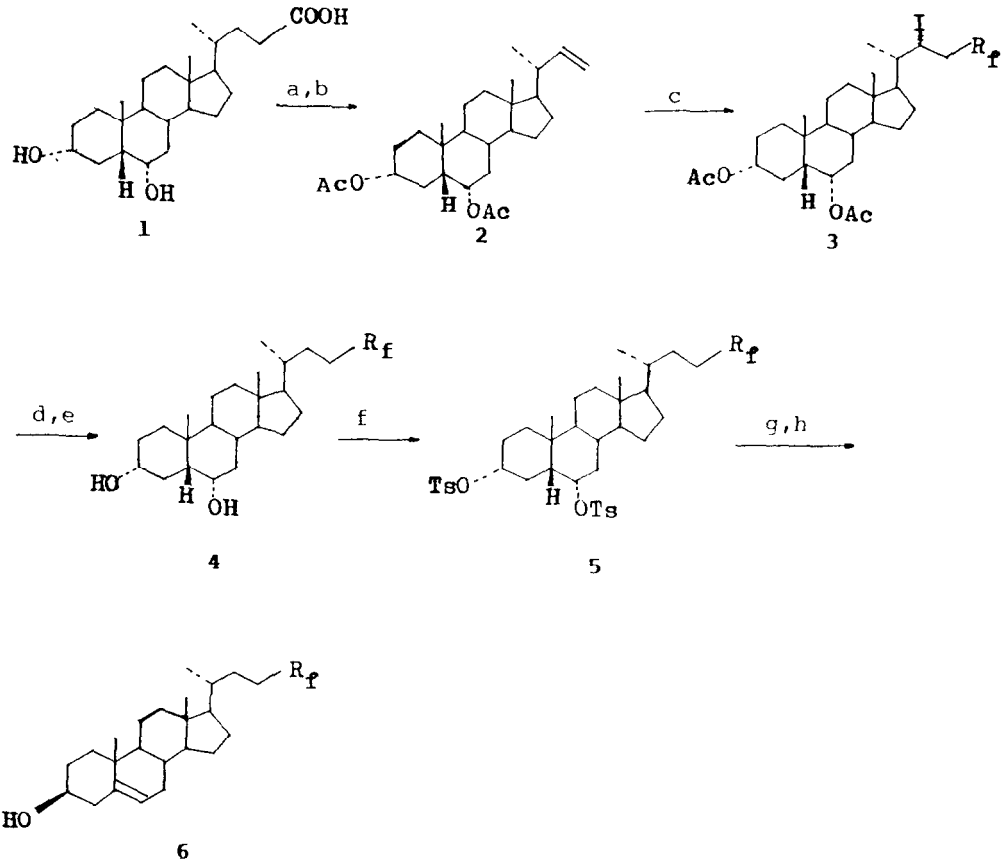
years [1]. Herz et al. [2] synthesized 26,27 fluorinated-Δ 24-cholesterol. 26,26,26,27,27,27-Hexafluoro-25-hydroxylcholesterol was synthesized by Piret et al. [3]. Recently, Sharts et al. [4] reported several methods for introducing perfluoroalkyl groups into steroid molecules. The present paper reports the preparation of cholesterol and its analogs with a fluorinated side-chain for the study of behavior of these fluorinated compounds toward the action of some microbial organisms.

RESULTS AND DISCUSSION

Starting from hyodeoxycholic acid (3α , 6α - dihydroxy-5β - cholanic acid **1**) , alkene **2** was prepared by a known method [5]. Sodium dithionite induced the addition of perfluoroalkyl iodides to **2** to give compound **3** in high yield. The Hunsdiecker reaction [6] was used to prepare 2-trifluoromethyl-perfluoropropyl iodide in good yield from silver 2-trifluoromethyl-perfluorobutanoate. Treatment of 2-trifluoromethylperfluorobutyl iodide with fuming sulfuric acid at 80°C [7] gave the corresponding 2-trifluoromethylperfluorobutanoyl fluoride. The carboxylic acid can also be obtained in good yield by transformation of 2-trifluoromethyl-perfluorobutyl iodide via the corresponding sodium 2-trifluoromethylperfluorobutanesulfinate [8] followed by treatment with hydrobromic acid [9]. Compound **3** was reduced with zinc dust to yield compound **4**. After the construction of ring A/B structure according to the known method [10], the title compounds were synthesized.

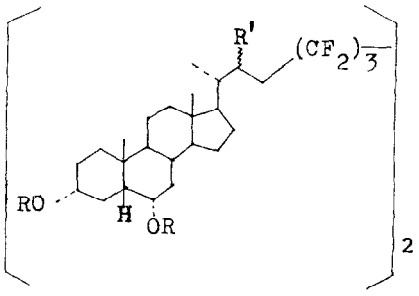


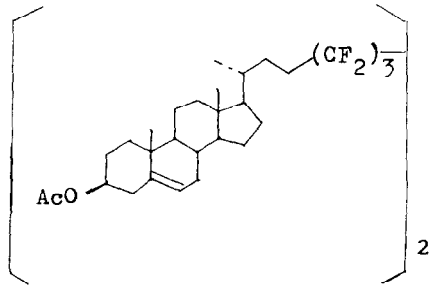
Scheme I

R_f: a, $CF_2CF(CF_3)_2$; b, $(CF_2)_2CF(CF_3)_2$; c, $(CF_2)_3CF_3$; d, $(CF_2)_4Cl$ e, $(CF_2)_6Cl$.

Reagents: a, $Ac_2O/AcOH$; b, $Pb(OAc)_4/Py$, C_6H_6 ; c, R_fI, $CH_2Cl_2/Na_2S_2O_4$, $NaHCO_3$, H_2O ; d, $Zn/(CH_3)_2CHOH$, concentrated HCl ; e, $KOH/EtOH$, H_2O ; f, TsCl/Py ; g, KOAc/DMF, H_2O ; h, $KOH/EtOH$, H_2O

In the addition of 1, 6-diiodo-perfluorohexane to alkene **2** , a mixture of mono- and bis-adducts, **3f** [R_f= $(CF_2)_6I$] and **7** were formed. By controlling the molar ratio of alkene **2** and the diiodide as 2:1 or 1:1, either **7** or **3f** can be obtained as the main product.





7 , R=Ac , R'= I

8a, R=Ac , R'= H

8b, R=H , R'= H

9 , R=Ts , R'= H

10

Reduction of compound **3f** with zinc dust caused the removal of both iodine atoms to give **4g**, [R_f=$(CF_2)_6H$], which was transformed in a similar way via **5g** to give **6g** . The bisadduct **7** was also transformed smoothly via **8b** and **9** to furnish the bis-sterol **10** . Preliminary microbiological assay showed that the side chain of compounds **6c, 6d** and **6e** were not degraded by Mycobacterium sp. which was able to degrade cholesterol side chains [11].

EXPERIMENTAL

The melting points are uncorrected. IR spectra were measured with Carl Zeiss 75 IR Spectrometer. ^{1}H NMR spectra were recorded on a Varian XL-200MHz spectrometer using TMS as internal standard, ^{19}F NMR spectra were recorded on Varian EM-360L spectrometer using TFA as an external standard. In ^{19}F NMR spectra, δ_{CFCl_3} (positive upfield) were calculated by $\delta_{CF_3COOH} + 76.8$. Mass spectra were recorded on a Finnigan-4021 spectrometer. The specific rotation was recorded on an Autopol III instrument. Silica gel HF of particle size 10-40 μ was used for column chromatography, under a positive pressure of 0.5 atmosphere.

The preparation of 2-trifluoromethyl-perfluoropropyl iodide

(1): With magnetic stirring, 100 g(0.25 mol) $(CF_3)_2CFCF_2CF_2I$ (purity 99%) was added dropwise to a solution of 250 ml fuming sulfuric acid at 80°C. The mixture was refluxed for five hours. The product was then distilled at the temperature of 50°C to give 50 g $(CF_3)_2CFCF_2COF$, yield 74%. b.p. 31°C. ^{19}F NMR δ -20.9(1F, t, COF), 74.1(6F, d, $2CF_3$), 114.1(2F, m, CF_2COF), 186.8(1F, m, CF).

(2): With magnetic stirring, 50g (0.188 mol) $(CF_3)_2CFCF_2COF$ was carefully added dropwise into 30 ml H_2O at 0°C. The mixture was stirred for further five hours. The organic phase was then separated and distilled under reduced pressure to give 40 g

$(CF_3)_2CFCF_2COOH$, yield 81%. ^{19}F NMRδ 72.3(6F, d, $2CF_3$), 111.8(2F, m, CF_2), 184.8(1F, m, CF).

(3): $(CF_3)_2CFCF_2COOAg$ was prepared by adding 10% excess of silver oxide (20 g, 0.086 mol) to a solution of 20 g (0.075 mol) $(CF_3)_2CFCF_2COOH$ in 20 ml H_2O. The reaction mixture was stirred and heated at 60°C for three hours. The silver 2-trifluoromethyl-perfluorobutanoate was soluble in ethyl ether, therefore the product was extracted with 100 ml ethyl ether. The ethereal layer was filtered to remove the suspended excess silver oxide. On evaporating the ether, 20 g of silver salt was collected, yield 71%.

(4): A 20 g (0.054 mol) sample of powdered silver 2-trifluoro-methylperfluorobutanoate was treated with 7.5 g (10% excess 0.059 mol) of powdered iodine at 100°C for five hours. The product was collected in a refrigerated trap to give 10 g $(CF_3)_2CFCF_2I$ [12], yield 54%. ^{19}F NMRδ 52.8(2F, d, CF_2I), 71.5(6F, d, $2CF_3$), 163.8(1F, m, CF).

Trifluoromethyl-perfluorobutanoic acid was also prepared as follows:

(1): Following the literature method [8], $(CF_3)_2CFCF_2CF_2I$ was readily transformed to $(CF_3)_2CFCF_2CF_2SO_2Na$, yield 90%. ^{19}F NMRδ 73.3(6F, d, $2CF_3$), 115.3(2F, m, CF_2), 52.3(2F, m, CF_2SO_2Na), 185.8(1F, m, CF).

(2): Following the known method [9], $(CF_3)_2CFCF_2CF_2SO_2Na$ was transformed to the corresponding carboxylic acid in 60% yield.

The preparation of compound **2**

Compound 2 was prepared from hyodeoxycholic acid according to established methods [5] .

The preparation of compound **3a**

The reaction was carried out under a nitrogen atmosphere. With magnetic stirring, 2 g (4.6 mmol) **2** , 10 ml CH_2Cl_2 , **2 g** (5.8 mmol)$(CF_3)_2CFCF_2I$, 50 ml H_2O were mixed. A 10 g sample of powdered $Na_2S_2O_4$ (C.P) was mixed with 5 g $NaHCO_3$, the mixture was divided into four portions, each portion was added into the reaction system at 50°C at five hours intervals. 30 ml CH_2Cl_2 was then added, the organic phase was separated and washed twice with water, dried with anhydrous Na_2SO_4 . After removal of the solvent the residue was chromatographed on a silica gel column using petroleum/acetone = 10:1 as eluant to give **3a** 3.2 g , yield 90%.

3a: ^1H NMRδ 2.8(2H, m, $C_{23}H_2$), 5.1(1H, m, $C_3\beta$-H), 4.7(1H, m, $C_6\beta$-H), 4.5(1H, m, C_{22}-H); ^{19}F NMRδ 72.8(6F, d, $2CF_3$), 106.8(2F, m, CF_2CH_2), 184.8(1F, m, CF); IR: 2950, 2880, 1740, 1300, 1240, 1180cm^{-1} .

3b, 3c, 3d, 3e were prepared similarly.

The ^{1}H NMR spectra of compounds **3b, 3c, 3d, 3e, 3f** and **7** are the same as that of 3a.

3b: ^{19}F NMR δ 72.8(6F, d, $2CF_3$), 113.3 (2F, m, CF_2CH_2), 116.8(2F, m, CF_2), 184.8(1F, m, CF); IR: 2950, 2880, 1740, 1320, 1240, 1180cm^{-1} .

3c: ^{19}F NMR δ 75.6 (3F, t, CF_3), 107.6(2F, m, CF_2CH_2), 118.7, 120.5 (4F, m, CF_2CF_2); IR: 2950, 2885, 1740, 1250, 1220, 1160cm^{-1}; m/e: 776(M^+); Analysis, Found: C, 46.40, H, 5.36, F, 21.80; $C_{31}H_{42}F_9IO_4$, Calc., C, 47.94, H, 5.45, F, 22.02 .

3d: ^{19}F NMR δ 66.2(2F, t, $ClCF_2$), 112.4(2F, m, CF_2CH_2), 117.8, 120.8(4F, m, $2CF_2$); IR: 2950, 2885, 1740, 1200, 1185, 1130cm^{-1}.

3e: ^{19}F NMR δ 66.1(2F, t, $ClCF_2$), 111.8(2F, m, CF_2CH_2), 118.3, 119.3, 121.5 (8F, m, $4CF_2$); IR: 2950, 2880, 1740, 1200, 1150, 1050 cm^{-1}; Analysis, Found: C, 44.82, H, 5.00, F, 24.76; $C_{33}H_{42}F_{12}ClIO_4$, Calc., C, 44.38, H, 4.74, F, 25.53 .

The preparation of 3f and 7

With magnetic stirring, 2 g (4.6 mmol) **2** , 10 ml CH_2Cl_2, 1.3g (2.3 mmol) $I(CF_2)_6I$, 50 ml H_2O were mixed. The reaction was conducted as above to give **7** 2.5 g yield 76%, **3f** 0.5 g, yield 11% .

7 : ^{19}F NMRδ111.3(4F, m, $2CF_2CH_2$), 119.3, 121.3(8F, m, $4CF_2$); IR : 2950, 2880, 1740, 1240, 1200, 1030cm^{-1}.

3f: ^{19}F NMRδ57.5 (2F, t, CF_2I), 111.8 (2F, m, CF_2CH_2), 118.8, 121.8 (8F, m, $4CF_2$); IR : 2950, 2880, 1740, 1240, 1200, 1140cm^{-1}.

The preparation of **4a**

With magnetic stirring, 0.75 g **3a** was dissolved in 20 ml isopropanol, 2 g zinc dust and then several drops of concentrated hydrochloric acid were added , the mixture was heated at 60°C for 2 hours. 50 ml ethyl ether and 50 ml H_2O were added , the ethereal layer was washed twice with water, and dried with anhydrous Na_2SO_4. After removal of ether the product was purified by chromatography on a silica gel column using petroleum/acetone = 5:1 as eluant to give **4a** 0.5 g , yield 93%.

4a: m.p. 184-185°C, $[\alpha]_D$, +6.9($CHCl_3$, C = 0.050); ^1H NMR δ4.1 (1H, m, $C_3\beta$-H), 3.6(1H, m, $C_6\beta$-H), 1.5 (2H, s, 2OH); ^{19}F NMR is the same as that of **3a**;IR:3390,2940,2880,1315,1290,1240,1050cm^{-1}; Analysis, Found: C, 56.74, H, 6.97, F, 30.19; $C_{27}H_{39}F_9O_2$, Calc., C, 57.23, H, 6.94, F, 30.18.

4b, 4c, 4d, 4e, 4f and **8a, 8b** were prepared similarly.

The ^1H NMR of **4b, 4c, 4d, 4e** and **8** are the same as that of **4a.**

4b: m.p. 170-172°C, $[\alpha]_D$, +7.4($CHCl_3$, C = 0.053); ^{19}F NMR is the same as that of **3b**; IR: 3390, 2940, 2880, 1330, 1280, 1240cm^{-1};

Analysis, Found : C, 55.37, H, 6.73, F, 31.92; $C_{28}H_{39}F_{11}O_2$, Calc., C, 54.54, H, 6.38, F, 33.89 .

4c: m.p. 140°C, $[\alpha]_D$, +5.3 ($CHCl_3$, C = 0.050); ^{19}F NMR is the same as that of **3c**; IR: 3390, 2940, 2880, 1250, 1160cm^{-1}; m/e : 548 (M^+-18), 530(M^+-36); Analysis, Found : C, 56.96, H, 6.76, F, 27.62 ; $C_{27}H_{39}F_9O_2$, Calc., C, 57.23, H, 6.94, F, 30.18 .

4d: m.p. 145°C, $[\alpha]_D$, +5.0($CHCl_3$, C = 0.053); ^{19}F NMR is the same as that of **3d**; IR: 3390, 2940, 2880, 1190, 1130cm^{-1} .

4e: m.p. 152-154°C, $[\alpha]_D$, +6.2($CHCl_3$, C = 0.062); ^{19}F NMR is the same as that of **3e**; IR : 3390, 2940, 2880, 1200, 1150, 1050cm^{-1} ;
Analysis, Found : C, 51.84, H, 5.49, F, 33.61 ; $C_{29}H_{39}F_{12}ClO_2$, Calc., C, 51.00, H, 5.76, F, 33.38 .

8a: m.p. 182°C, ^1H NMR δ2.8(4H, m, $2C_{23}H_2$), 5.1(2H, m, $2C_3\beta H$), 4.7(2H, m, $2C_6\beta$-H), 2.0(12H, s, $4CH_3COO$-); ^{19}F NMR is the same as that of **7** ; IR : 2950, 2880, 1740, 1240, 1200, 1140cm^{-1}; Analysis, Found: C, 61.86, H, 7.77, F, 19.67; $C_{60}H_{84}F_{12}O_8$, Calc., C, 61.95 , H, 7.22, F, 19.60 .

8b: ^{19}F NMR is the same as that of **7**; IR : 3400, 2950, 2880, 1240, 1200, 1030cm^{-1} .

4g: ^1H NMR δ4.1(1H, m, $C_3\beta$-H), 3.6(1H, m, $C_6\beta$-H), 1.5 (2H, s, 2OH), 6.35,6.10, 5.85(1H, t, t, CF_2CF_2H, J_{H-F} = 50Hz); ^{19}F NMR δ111.8(2F, m, CF_2CH_2), 119.8, 121.3 (6F, m, $3CF_2$), 127.5

(2F, m, CF_2CF_2H), 134.8(2F, d, CF_2H, J_{F-H}= 50Hz); IR : 3400, 2940, 2880, 1240, 1200, 1140cm^{-1}.

The preparation of **5a**

About 0.5 g **4a** and 2 g tosyl chloride were dissolved in 20 ml pyridine. The mixture was allowed to stand at room temperature for two days, after which, 100 ml cold water was slowly added. The solution was acidified with excess dilute sulfuric acid and extracted with 2×50ml ethyl ether. The ethereal layer was washed with sodium bicarbonate solution and water and dried over anhydrous Na_2SO_4. After removal of the solvent, the product was recrystallized from MeOH-EtOH to give **5a** 0.74 g., yield 98%. ^1H NMRδ 7.8(4H, m, $-C_6H_4-$), 7.4 (4H, m, $-C_6H_4-$), 2.5 (6H, s, 2aryl-CH_3), 4.8(1H, m, $C_3\beta$-H), 4.3(1H, m, $C_6\beta$-H); ^{19}F NMR is the same as that of **3a**; IR: 2960, 2880, 1600, 1460, 1360, 1300, 1250, 1100 cm^{-1} .

5b, 5c, 5d, 5e, 5g and **9** were prepared in a similar way, their ^1H NMR and IR were almost the same as that of **5a** and ^{19}F NMR is the same as that of **3b, 3c, 3d, 3e** and **4g** respectively.

The preparation of **6a**

With magnetic stirring 0.7 g **5a** and 5 g KOAc were added to a solution of 30 ml DMF and 5 ml H_2O. The mixture was heated at 110°C for 5 hours, after which 100 ml H_2O was added. The mixture

was extracted with 3×30 ml ethyl ether and the ethereal layer washed twice with water. After removal of ether, the residue was dissolved in 20 ml EtOH. Then 2 ml H_2O and 3 g KOH were added and the mixture refluxed for 3 hours. The hydrolyzed solution was added to 100 ml H_2O and the solution extracted with 2×50 ml ethyl ether. The ethereal layer was dried with anhydrous Na_2SO_4. After removal of the solvent the product was purified by chromatography on a silica gel column using petroleum/ acetone = 6:1 as eluant to give **6a** 0.33 g yield 70% .

6a: m.p. 170°C, $[\alpha]_D$, -29.5($CHCl_3$, C = 0.051); ^1H NMRδ5.3 (1H, m, C_6-H), 3.5(1H, m, $C_3\alpha$-H), 2.3(2H, m, $C_{23}H_2$); ^{19}F NMR is the same as that of **3a**; IR : 3420, 2980, 2950, 2900, 2860, 1670, 1320, 1290,1240 cm^{-1}; m/e : 548(M^+), 530(M^+-18), 515, 463, 437 ; Analysis, Found: C, 59.27, H, 6.63, F, 31.03 ; $C_{27}H_{37}F_9O$, Calc., C, 59.11, H, 6.80, F, 31.17 .

6b, 6c, 6d, 6e, 10 and 6g were prepared similarly.

6b: m.p. 138°C, $[\alpha]_D$, -30.4 ($CHCl_3$, C = 0.028); ^1H NMR is the same as that of **6a** ; ^{19}F NMR is the same as that of **3b**; IR: 3420, 2980, 2950, 2860, 1670, 1300, 1240 cm^{-1}; m/e: 598(M^+), 580(M^+-18), 565, 513, 487; Analysis, Found: C, 57.43, H, 6.54, F, 31.00 ; $C_{28}H_{37}F_{11}O$, Calc., C, 56.18, H, 6.23 , F, 34.91 .

6c: m.p. 138°C, $[\alpha]_D$, -29.8($CHCl_3$, C = 0.036); ^1H NMR is the same as that of **6a**; ^{19}F NMR is the same as that of **3c**; IR: 3420, 2950, 2860, 1670, 1240, 1200cm^{-1}; m/e: 548(M^+), 530(M^+-18); Analysis, Found: C, 59.55, H, 6.75, F,31.47; $C_{27}H_{37}F_9O$, Calc., C, 59.12, H, 6.80, F, 31.17.

6d: m.p. 138-139°C, $[\alpha]_D$, -27.9($CHCl_3$, C = 0.067); ^1H NMR is the same as that of **6a**; ^{19}F NMR is the same as that of **3d**; IR: 3420, 2980, 2950, 2860, 1670, 1200, 1195 cm^{-1}; m/e: 564(M^+), 546(M^+-18); Analysis, Found: C, 57.48, H, 6.62, F, 26.91, Cl, 5.68; $C_{27}H_{37}F_8ClO$, Calc., C, 57.40, H, 6.60, F, 26.90, Cl, 6.28.

6e: m.p. 150°C, $[\alpha]_D$, -30.4($CHCl_3$, C = 0.063); ^1H NMR is the same as that of **6a**; ^{19}F NMR is the same as that of **3e**; IR: 3420, 2980, 2860, 1670, 1200, 1150cm^{-1}; m/e : 664(M^+), 645(M^+-19); Analysis, Found: C, 51.47, H, 5.50, F, 34.48; $C_{29}H_{37}F_{12}ClO$, Calc., C, 52.37, H, 5.57, F, 34.31 .

10: m.p. 202°C, $[\alpha]_D$, +14.0 ($CHCl_3$, C = 0.030); ^1H NMR δ 5.2 (2H, m, C_6-H), 4.7(2H, m, $C_3\alpha$-H), 2.0(6H, s, CH_3COO-); ^{19}F NMR is the same as that of **7**; IR: 2980, 2950, 2860, 1740, 1670, 1240, 1200, 1030cm^{-1}; Analysis, Found: C, 62.35, H, 7.54, F, 20.57; $C_{56}H_{78}F_{12}O_4$, Calc., C, 64.47, H, 7.54, F, 21.85 .

6g: m.p. 146-148°C, $[\alpha]_D$, -28.6($CHCl_3$, C = 0.018); ^1H NMR δ6.35, 6.10, 5.85(1H, t, t, CF_2CF_2H, J_{H-F} = 50Hz), 5.3(1H, m, C_6-H), 3.5(1H, m, $C_3\alpha$-H), 2.3(2H, m, $C_{23}H_2$); ^{19}F NMR is the same as that of **4g** IR : 3420, 2980, 2950, 1670, 1240, 1200, 1140cm^{-1}; m/e : 630(M^+), 612(M^+-18); Analysis, Found: C, 54.92, H, 6.31, F, 34.07; $C_{29}H_{38}F_{12}O$, Calc., C, 55.23, H, 6.07, F, 36.15 .

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